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(54) Title: METHODS AND ARTICLES FOR ENHANCED PROTEIN ADSORPTION AND FLUID MANAGEMENT ON SURFACES

(57) Abstract

A method for preparing test articles comprises adsorbing a protein onto a solid phase surface, where the surface has been pretreated by plasma etching. Usually, the pretreatment step will comprise etching of the solid phase surface in a conventional plasma etch reactor. Such pretreatment of the solid phase reduces the time necessary to achieve adequate protein adsorption and enhances the protein adsorption in the presence of denaturing conditions, such as detergents. Such pretreatment will also enhance hydrophobicity of naturally hydrophilic materials to limit fluid flow on the surface, usually through flow paths, chambers, and the like.

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METHODS AND ARTICLES FOR ENHANCED PROTEIN ADSORPTION AND FLUID MANAGEMENT ON SURFACES

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to the production of test articles used for determining the presence of analyte in biological samples. More particularly, the present invention relates to a method for treating test articles to promote protein adsorption and in some cases to enhance surface hydrophobicity to provide fluid control in the article.

A wide variety of assay techniques and methods exist for determining the amount of a target analyte in a biological or other sample. Specific binding assays rely on detecting an analyte using a specific binding substance which reacts with the analyte in a highly selective manner. Numerous protocols and formats for performing such specific binding assays are described in the patent, technical, and medical literature.

Of particular interest to the present invention, many specific binding assays employ a solid phase surface having a protein specific binding substance, such an antibody, antibody fragment, avidin, or a derivatized protein carrier, immobilized on the surface. The surface may then be utilized to capture analyte through direct or indirect binding of the immobilized specific binding substance to the target analyte.

Such solid phase surfaces used for performing immunoassays are frequently the most costly component in a commercial immunoassay kit. Procedures and protocols for immobilizing proteins onto a solid phase often require multiple steps and are thus time-consuming and expensive. While the cost can be reduced by employing batch manufacturing processes, the batch size (and hence cost advantage) is

limited by the need to carefully control the characteristics of each solid phase being produced. Thus, there is a general need in the immunoassay industry to provide processes for producing solid phase test articles at a reduced cost.

Heretofore, protein immobilization onto solid phase test surfaces has been achieved by a variety of techniques. For example, covalent immobilization of proteins provides for irreversible binding (*i.e.*, with minimal or no desorption of protein), but suffers from the need to derivatize the solid phase surface and/or the protein and to include the additional fabrication steps necessary for effecting covalent binding. As an alternative to covalent immobilization of proteins, the fabrication of many immunoassay solid phase articles relies on non-covalent protein adsorption to a hydrophobic surface. While such protein adsorption protocols are relatively simple, strong attachment of the protein to the surface requires relatively long protein incubation times, typically on the order of hours. Without such prolonged incubations, desorption of many protein reagents from a conventional surface will occur, particularly when the surface is exposed to detergents and other denaturing reagents during an assay protocol. The need to utilize a prolonged adsorption step during the manufacture of an immunoassay solid phase article is undesirable since it increases the manufacturing cost.

In addition to concerns over protein immobilization, methods for preparing test articles for use in immunoassays must also address the need to control fluid flow over the surfaces thereof. In many cases, such as with microtiter wells, it is desirable that introduced fluids spread evenly over the surface in order to contact all portions thereof. In other devices, such as analytical rotors, it may be desirable to limit the flow of fluid within a chamber or flow path thereon prior to rotation of the rotor in order to positively effect flow. That is, it will sometimes be desirable to initially introduce a fluid and have that fluid contained within a restricted region on the rotor prior to transfer to another region. While some materials, such as polypropylene, are naturally hydrophobic (and thus inhibiting to the flow of

aqueous media), others such as acrylates, are generally more hydrophilic and must be modified to impart a hydrophobic nature to the surface in order to limit the flow of aqueous solutions.

For these reasons, it would desirable to provide improved methods for non-covalently immobilizing protein materials onto solid phase surfaces to produce test articles useful for performing immunoassays and other biological assays. In particular, it would be desirable to provide improved fabrication methods for adsorbing proteins onto a surface, where enhanced binding with decreased desorption can be achieved in relatively short periods, preferably with protein incubation times below 1 hour, more preferably ½ hour, and even more preferably below 10 minutes. It would be further desirable to be able to impart a hydrophobic nature to surfaces which are naturally rather hydrophilic, such as acrylates, in order to control the flow characteristics of aqueous solutions on solid phase surfaces of test articles intended for immunoassays and other biological assays. It would be particularly desirable if such improvements could be achieved by the same surface treatment protocols used to enhance protein adsorption.

2. Description of the Background Art

U.S. Patent No. 5,316,784, describes a two-step process for attaching immunologically active substances, such as antibodies, antigens, and binding proteins, to solid phase surfaces by applying a mixture of the active substance and a linking group having photoactivable groups to the surface. After allowing the mixture to absorb into the surface, the active substance is covalently attached by activating the linking group. U.S. Patent No. 5,258,041, describes the use of spacer arms having hydrophobic guiding groups for attaching biomolecules to solid phase supports.

The inventors of the present application have filed an earlier application with a third co-inventor on related subject matter. See, application serial no. 08/374,265, filed

on January 18, 1995, the full disclosure of which is incorporated herein by reference.

SUMMARY OF THE INVENTION

The present invention provides an improved method for the non-covalent immobilization of proteins to a solid phase surface to produce articles which are particularly useful in the performance of immunoassays and other biological procedures. The improvement comprises plasma etching with a reactant gas and under conditions selected to provide such enhanced protein binding. While the mechanism is not understood in detail, it is presently believed that the reactant gas chemically modifies the surface and/or leaves residual moieties on the surface which promote the desired binding via one or more binding forces, including hydrophobic hydrogen bonding, Van der Waals forces, and other interactions. Exemplary reactant gases include hydrocarbons (e.g., CH_4 and C_2H_6), halocarbons (e.g., CF_4, CCl_4, CF_3Cl, CF_3Br), other halogen-containing gases (e.g., S_2F_6), and combinations thereof. Preferred is the use of halocarbons, with the most preferred gas being carbon tetrafluoride (CF_4), and exemplary conditions include a treatment time in the range from 1 to 20 minutes, a treatment pressure in the range from 0.1 to 1 Torr, at a power level from 100 W to 1000 W. It has been found that such pretreatment to enhance protein binding allows the use of very short protein adsorption times, typically below 1 hour, preferably below ½ hour, and usually below 10 minutes, while still achieving very strong binding which resists desorption, even when exposed to detergents under mildly denaturing conditions.

The present invention further provides for improving the management of the flow of aqueous solutions on the surfaces of test articles, particularly those used in immunoassays and other biological assays. In particular, it has been found that plasma treatment as described above can enhance surface hydrophobicity on surfaces which are generally rather hydrophilic in their native (unmodified) state, such as acrylates, e.g., methylmethacrylate surfaces. The methods

comprise a solid phase surface having at least one flow path or test chamber formed therein. The solid phase surfaces will typically comprise naturally hydrophilic organic polymers, such as acrylates, which have been molded or machined to include surface cavities which define the flow paths and/or test chambers. The flow paths and test chambers will be treated in order to enhance their hydrophobicity. Such enhanced hydrophobicity, in turn, will limit the flow of aqueous fluids through such flow paths and within such test chambers. Thus, such procedures will be particularly useful in the preparation of analytical rotors, where it is desired to be able to introduce or transfer aqueous fluids to the rotor and have the fluids remain within a fixed location on the rotor prior to transfer, usually by rotation of the rotor above a desired threshold rotational speed. The methods of the present invention for imparting enhanced hydrophobicity to flow paths and test chambers, however, will find use with any test article where it is desired to be able to selectively effect fluid transfer within the test article and through such flow paths and test chambers, usually by application of an outside force to the fluid. The preferred method for imparting a greater degree of hydrophobicity is plasma treatment according to the methods described above.

The present invention further provides improved test articles prepared by the methods described above.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic illustration of a test article which may be prepared using the methods of the present invention.

Fig. 2 is a schematic illustration of a plasma treatment apparatus useful for performing a method according to the principles of the present invention.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Methods are provided for attaching proteins to solid phase surfaces to form test articles useful for detecting target analytes in biological and other assays. The methods

of the present invention are in some cases also useful for modifying the flow characteristics of flow paths and test chambers on such solid phase surfaces in order to improve the usefulness of the test articles in performing immunoassays. In particular, by plasma etching the entire solid phase surface or selected portions thereof, both protein attachment and flow management of test liquids and reagents may be improved.

The target analyte will usually be a member of a specific binding pair (SBP), including compounds, compositions, aggregations, and virtually any other substance which may be detected or reacted by immunological or equivalent techniques. That is, the analyte, or a portion thereof, will usually be antigenic or haptenic, defining at least one epitopic site, or will be a member of a naturally-occurring binding pair (*e.g.*, carbohydrate and lectin, hormone and hormone receptor, ligand and anti-ligand, and the like). Analytes of particular interest include antigens, antibodies, proteins, glycoproteins, carbohydrates, macromolecules, toxins, bacteria, tumor markers, and the like, which define a plurality of epitopic sites. Other analytes of interest include haptens, drugs, and other small molecules, which usually define only a single epitopic binding site. A non-exhaustive list of exemplary analytes is set forth in U.S. Patent No. 4,366,241, at column 19, line 7, through column 6, line 42, the disclosure of which is incorporated herein by reference. Of particular interest to the present invention is the detection of cardiac markers, such as troponin I, troponin T, myoglobin, and creatine kinase isozymes.

The analytes may be present in a wide variety of samples, where the sample is liquid, can be liquified, or can be suspended in a liquid. The methods of the present invention will find their greatest use with biological specimens such as blood, serum, plasma, urine, cerebral fluid, spinal fluid, ocular lens liquid (tears), saliva, sputum, semen, cervical mucus, scrapings, swab samples, and the like, which are frequently employed in the diagnosis and monitoring of disease and therapeutic treatments. In addition, the

methods of the present invention may be used with industrial, environmental, and food samples, such as water, process streams, milk, meat, poultry, fish, conditioned media, and the like. In certain circumstances, it may be desirable to pretreat the sample, such as by liquification, separation, solubilization, concentration, filtration, chemical treatment, or a combination of these steps, in order to improve the compatibility of the sample with the remaining steps of the assay, as described hereinafter. The selection and pretreatment of biological, industrial, and environmental samples prior to immunological testing is well known in the art and need not be further described. The samples will usually be in the form of an aqueous solution, suspension, or the like. As will be discussed in more detail below, the flow characteristics of such aqueous media can be advantageously controlled on the solid phase surfaces which are prepared according to the method of the present invention.

Exemplary proteins which may be adsorbed onto the modified surfaces of the present invention include those which are conventionally attached to unmodified surfaces, *e.g.*, immunoglobulins, avidin, streptavidin, albumins, thyroglobins, ferritin, and the like.

The adsorbed proteins may be directly useful in the immunoassays, *i.e.*, they may comprise the cognate member of a specific binding pair which includes the analyte of interest. Examples of such directly useful proteins include antibodies raised against the analyte, receptor proteins used to detect hormones and other small molecules, and the like. Alternatively, the adsorbed protein may comprise a carrier molecule attached to a hapten or other binding moiety selected to participate in a particular binding protocol. Typically, the haptens will provide for high affinity binding to another molecule, where the hapten-binding molecule pair provides an intermediate binding reaction in the assay protocol. Exemplary haptens which may be attached, usually covalently attached, to the surface-adsorbed protein of the present invention include biotin (which binds to avidin, streptavidin, and anti-biotin antibody with very high affinity), fluorescein

(which binds with high affinity to anti-fluorescein antibody), dinitrophenol (which binds with very high affinity to anti-dinitrophenol antibody), digoxin (which binds with very high affinity to anti-digoxin antibody), luminol (which binds with very high affinity to anti-luminol antibody), theophylline (which binds with very high affinity to anti-theophylline antibody), morphine (which binds with very high affinity to anti-morphine antibody), and the like. The preparation of hapten-protein conjugates which could be used in the methods and articles of the present invention are well described in the patent and scientific literature. Specific techniques are described in copending application serial no. 08/374,265, the full disclosure of which has previously been incorporated herein by reference.

The surface to be treated and modified to adsorb proteins according to the method of the present invention may be composed of any material that can be formed into a test article or device having a reaction zone, including organic polymers, glass, ceramics, metals, and the like. The surface may be composed of an organic polymer having a natural hydrophilic surface (*i.e.*, a surface which is hydrophilic (has a small water contact angle, usually below 100°) prior to treatment according to the present invention), such as an acrylate, in which case, plasma etching will enhance protein binding and will also impart hydrophobicity to modify the surface flow characteristics. Alternatively, the material may be naturally more hydrophobic, e.g., polystyrene, in which case plasma etching only enhances protein binding. Particularly preferred materials for forming a solid phase surface according to the present invention are methylmethacrylate polymer and polystyrene.

The surface will usually be formed on or as a part of a substrate which is at least part of a test article which may be employed in an immunological or other assay protocol. Exemplary substrate configurations include disks, strips, plates, tubes, wells, particles, microspheres, magnetic particles, and the like. Usually, the substrate will be composed entirely of the surface material, but this is not

necessary. The substrate may also be formed by coating a desired organic polymer or other material over a portion thereof, by forming a laminate, composite, or other structure comprising two or more materials. At least a portion of the solid phase surface will be treated according to the method of the present invention to enhance binding prior to protein adsorption. Optionally, two or more discrete regions or zones on the surface may be treated where it is desired to adsorb protein only within such zones. An exemplary disk substrate which could be prepared using the method of the present invention is described in copending application serial no. 08/374,265, the full disclosure of which has previously been incorporated herein by reference. It will be appreciated that the treatment method of the present invention could be utilized as an improvement in the method of this earlier application.

The surface to be treated according to the present invention may also include flow paths, test chambers, and other fluid-receiving geometries thereon. In some cases, it will be desirable to be able to control or manage fluid flow or transfer between such chambers, flow paths, and the like. In particular, it may be desirable to be able to introduce an aqueous fluid into an inlet chamber and maintain the fluid therein until application of an external force to effect fluid transfer. The most common situation in which this will occur is with the use of analytical rotors of the type described in copending application serial no. 08/521,615, the full disclosure of which has previously been incorporated herein by reference. In such cases, fluid retention and management can be enhanced by imparting a hydrophobic surface to such chambers, flow paths, and other portions of the test surface which will contact the liquid. The hydrophobic surface will cause the aqueous test sample, reagent, or other fluid, to bead (have a water contact angle in the range from 100° to 120°) on the surface and not flow from the point of application until the external force is applied, *e.g.*, through rotation of an analytical rotor.

An exemplary rotor 10 is illustrated in Fig. 1. The rotor includes inlet chambers 12, 14, and 16, which are connected to a test chamber 18 by flow paths 20, 22, and 24, respectively. It will be appreciated that by treatment of the inlet chambers 12, 14, and 16, as well as the flow paths 20, 22, and 24, any aqueous fluid which are introduced to the inlet chambers will tend to bead up and remain within the inlet chambers until the rotor is rotated to effect transfer to the test chamber 18. The test chamber 18 also illustrates reaction zones 30, 32, 34, and 36, each of which may include an adsorbed protein, where protein adsorption is enhanced by the method of the present invention as described above. Further details of the construction of the exemplary rotor 10 are described in copending application serial no. 08/521,615, the full disclosure of which has previously been incorporated herein by reference.

The solid phase surface may be treated by a variety of etching techniques, particularly including dry etching techniques. Suitable dry etching techniques include plasma etching, reactive ion etching, and ion milling, where the surface is exposed to highly reactive chemical species produced by energizing a gas or other ion source. Particularly preferred are plasma etching techniques where a very low pressure reactant gas, typically having pressure in the range from 0.1 to 1 Torr, is exposed to radio frequency energy to produce ions, free radicals, and neutral species, all having high kinetic energies. Preferred reactive gases include hydrocarbons, such as CH_4 and C_2H_6; halocarbons, such as CF_4, CCl_4, CF_3Cl, and CF_3Br; and other halogen-containing gases, such as S_2F_6.

The RF energy will be applied at a power level in the range from 1 W to 1000 W, for a time sufficient to impart the desired level of hydrophobicity, usually from 1 minute to 20 minutes, preferably from 1 minute to 10 minutes. Plasma etching may be performed in conventional plasma reactors, such as reactor 10 illustrated in Fig. 2. The process gas is continually introduced through an inlet 12 and flows between electrodes 14 and 16 to produce a glow discharge within the

reactant gas. Process gas is withdrawn through an exit port 18 to a vacuum system which maintains the desired low pressure level within the reactor 10. Reactor 10 as illustrated is an isotropic reactor, where etching occurs at a uniform rate in all directions. Thus, the substrate 20 which is treated within the reactor 10 may be oriented in any convenient orientation. Alternatively, it would be possible to perform plasma etching according to the present invention using an anisotropic etcher, typically a parallel plate etcher, where the substrate would be oriented in a parallel manner between opposed plates.

A preferred plasma etching protocol comprises initial plasma etching with an inert gas, such as Argon, followed by etching with the hydrocarbon or halogen-containing reactant gas.

After the solid phase surface, or portion thereof, has been plasma-etched, protein will be adsorbed to the surface in the generally conventional manner. The adsorption process, however, will generally be much shorter than that required by otherwise equivalent processes where the surface has not been pretreated by plasma etching. That is, it will require much longer protein adsorption times in order to achieve an equivalent strength of protein adsorption on surfaces which have not been treated to enhance hydrophobicity. Moreover, even prolonged adsorption times will not always achieve an equivalent strength of protein adsorption. As is further shown in the Experimental section hereinafter, the pretreatment methods of the present invention can provide protein adsorption having a higher resistance to desorption under denaturing conditions than that achieved with conventional adsorption procedures, regardless of the length of time of the adsorption step.

Generally, protein adsorption will comprise applying a liquid medium containing the protein of interest to the solid phase surface, by a variety of techniques, including pipetting, dipping, spraying, inkjet printing (as described in detail in copending application serial no. 08/374,265, the full disclosure of which has previously been incorporated

herein by reference), and the like. The present invention generally allows for protein adsorption to occur at room temperature, for relatively short period of time, usually less than 1 hour, preferably in the range from 1 minute to 20 minutes, more preferably in the range from 2 minutes to 10 minutes. After adsorption, the surface will usually be washed to remove non-bound proteins and other contaminants. After such washing, the solid phase surfaces will generally be ready for use in an immunoassay or other biological test procedure.

Surfaces having proteins adsorbed as described above may, however, be subjected to additional treatment in order to even further enhance binding. For example, proteins may be heated to a relatively high temperature, often above 50°C, sometimes above 90°C, or higher, to accelerate and further enhance adherence of the protein to the surface. Such heat treatment methods will generally only be possible with hapten-protein carrier combinations, where the hapten is not denatured by the heating. It will also be possible to enhance non-covalent binding by covalently cross-linking the proteins to the solid phase surface by subjecting the proteins and surface to suitable conditions, *e.g.*, light, radiation, heat, cross-linking reagents, or the like. Again, such additional attachment is generally only possible with hapten-protein carrier combinations where the hapten is not degraded by the conditions. Such further attachment procedures are described in more detail in copending application serial no. 08/374,265, the full disclosure of which has previously been incorporated herein by reference.

The following examples are offered by way of illustration, not by way of limitation.

Experimental

1. Immobilizing Biotinylated BSA or Plasma-etched Acrylic Test Articles

A. Initial Acrylic Disk Preparation

35 mm (dia.) methacrylate disks used for substrate material were obtained from the Germanow-Simon company. They were machined from Hesalite HTC material and were 1.1 or 1.4 mm thick. This material has optical properties that are sufficient for interferometric, fluorescent, or other types of interrogation. Disks were shipped from the vendor laminated on both sides to minimize scratching during machining, transport, and storage. Prior to plasma etching, protein adsorption and assay, laminates were removed, and the disks were rinsed with deionized water and in some instances washed with mild detergents both to quench static electricity build-up and to remove debris remaining from the machining process.

B. Acrylic Disk Surface Modification Through Plasma Etching

Plasma etching with various gases was used to modify the surface characteristics from those of native acrylic, either in the direction of a more hydrophilic surface or a more hydrophobic one. Etchants included CO_2, (Plasmaline lab etcher, 10 min exposure, 200-300 W forward RF, 0.1-0.5 Torr), H_2O vapor (Gasonics Pilot Test Etcher, 5 min exposure, 600 W, 0.4 Torr), CF_4 (Gasonics Pilot Test Etcher, 10 min exposure, 600 W, 0.35 Torr), and a $H_2(5\%)/N_2$ combination (Gasonics Pilot Test Etcher, 10 min exposure, 600@, 0.35 Torr). Etching with CO_2, H_2O vapor, and the $H_2(5\%)/N_2$ combination enhanced the hydrophilicity of the acrylic surface, decreasing the H_2O contact angle. Etching with CF_4, in contrast, enhanced the hydrophobicity of the plastic surface, markedly decreasing the H_2O contact angle. A suitable system for performing plasma etching is illustrated in Fig. 2.

C. Preparation of Biotinylated BSA Solutions

Bovine serum albumin (BSA) was covalently attached to biotin moieties (biotinylated) starting with 60 mg of a 10 mg/mL solution of BSA in phosphate buffered saline (PBS, 10 mM, pH 7.4). The BSA was then conjugated at a molar ratio

of ca. 10:1 NHS-LC-biotin to BSA. For 60 mg of BSA, 4.1 mg of NHS-LC-Biotin (Pierce #94052374) was dissolved in 60 μL of DMF (Pierce #931026155) and added to the 6 mL of BSA in PBS. This reaction mixture was placed in an ice bath for 2 hours followed by placing the mixture in a 12,000 MWCO dialysis tubing and dialyzing against 0.1 M phosphate buffer (pH 7.0, with at least two 1 L changes) at 4°C to remove unreacted biotin. Following dialysis and hapten number assessment, 0.1% NaN_3 was added to the concentrated biotin-BSA (B-BSA) to discourage microbial growth. Hapten numbers in the range from 2 to 9 were obtained.

D. Application of Biotinylated BSA solutions to Acrylic Test Articles

Before applying these solutions for adsorption, an aliquot of a concentrated B-BSA solution was diluted to 40 μg/mL in pH 7.4 PBS and degassed.

E. Washing Protein-coated Articles to Remove Excess Materials

Excess buffer/protein materials were washed from protein coated disks with PBS/0.1% Tween® 20 (Mallinckrodt) so that only an adsorbed monolayer of B-BSA was left on the acrylic surface for subsequent chemistry steps. This washing process removed most of the protein that was originally spotted (ca. 99%).

F. Retention of Biotinylated BSA on Plasma-modified Acrylic Test Articles

To characterize the binding process and enhance retention of proteins to the acrylic test surface, the influence of plasma modification of the acrylic test surfaces on protein binding kinetics was examined. Table 1 contains data illustrating that CF_4 plasma etching (which renders the acrylic test surface more hydrophobic) both (1) enhances binding at short contact times (ca. 5 minutes) to a level that is normally achievable on a non-etched disk only after a much longer contact time and (2) renders the binding effectively irreversible, *i.e.*, resistant to desorption. In contrast, the etching conditions that increase surface hydrophilicity (CO_2, H_2O vapor, and the $H_2(5\%)/N_2$ combination have the opposite effect as the (1) decrease short term binding, (2) lower the resistance to overnight desorption, and (3) apparently lower total binding capacity.

Materials

Biotinylated-BSA: see "C. Preparation of Biotinylated BSA Solutions" above. This material was diluted to 40 μg/mL in PBS before use.

Assay Diluent: 0.1% BSA (Pentax), 0.05% Tween® 20 (Mallinckrodt) in 10 mM PBS, 0.2 μm filtered (Gelman acrodisc).

Wash Buffer: 0.1% Tween® 20 (Mallinckrodt) in 10 mM PBS, 0.2 μm.

SA-HRP Conjugate: Streptavidin-horse radish peroxidase (SA-HRP, Pierce, Product #21127) was reconstituted to 1 mg/mL and frozen. Prior to use, the stock was further diluted in the 1:1000 to 1:1000000 range with Assay Diluent.

o-Phenylenediamine Dihydrochloride (OPD) substrate: A solution of 0.4 mg/mL OPD was made by dissolving a 15 mg tablet of OPD (Sigma) into 37 mL of 0.1 M citrate-phosphate buffer, pH 5.0. Immediately prior to use, 10 mM hydrogen peroxide was added (37 μL of 30% solution, Sigma) as an activating agent. Sulfuric acid (2N) was added in an equal volume to OPD to stop the reaction at the desired time.

Rings: Teflon rings (themselves plasma etched with CO_2 to enhance binding to the double stick tape) several millimeters thick and 0.25 inches in inner diameter were affixed to the clean, unspotted, 35 mm acrylic test articles (Germanow-Simon) with double-stick tape to facilitate containment of liquid materials above the test surface. After the protein adsorption step in the assay, the rings were removed and a fresh ring and double-stick tape was affixed to the original double-stick tape. This eliminates assay activity due to "wall effects."

Procedure

1. Acrylic test articles, either virgin or those previously etched with one of the test etchants, with teflon rings affixed had 100 μL of biotinylated BSA added to each well and contacted for either 5 minutes or overnight (ca. 12 hours).

2. One half of the 5 minute contact test articles were washed for 10 minutes in wash buffer (2 L for 10 disks) in an automated washer rotating at 46 rpm to remove excess biotinylated BSA and then the wells were rinsed several times manually with deionized water and stored for assay. The other half of the 5 minute contact disks were washed for an additional 12 hours with Wash Buffer in the automated washer (46 rpm) to stimulate desorption. After this stress test, the test articles were also rinsed several times with deionized water, dried, and stored.

3. The remaining 1/3 of the disk lot was stored overnight with the biotinylated BSA solutions still in contact with the disk surface. They were then washed for 10 minutes in wash buffer in the automated washer rotating at 46 rpm to remove excess biotinylated BSA and then the wells were rinsed several times manually with deionized water and dried.

4. All teflon rings were then replaced with new ones and a new piece of double-stick tape to eliminate any biotin BSA except for that bound to the test articles surface.

5. The SA-HRP conjugate (at a 1:50000 dilution) was then added to the wells on all the disks in a 100 μL volume.

6. After 60 minutes, excess SA-HRP was removed from the wells and the wells were then washed with a back-and-forth motion in two cups of wash buffer (several strokes in each cup).

7. The freshly-prepared OPD substrate was then added to the wells (75 μL) and allowed to react for 10 minutes without agitation.

8. The reaction was stopped by adding 75μL of 2 N H_2SO_4.

9. A Molecular Devices microplate reader (E_{MAX}) was used to determine absorbance at 405 nm after 140 μL of the reaction mixture from each teflon well was transferred to the microplates.

The results are set forth in Table 1.

TABLE 1: BIOTINYLATED BSA ADSORPTION/DESORPTION WITH PLASMA ETCHED ACRYLIC

	Streptavidin-HRP Binding (A 405 nm)				
Plasma Treatment:	none	CO_2	H_2O	H_2/N_2	CF_4
1) 5 min. adsorption	0.434	0.257	0.216	0.253	0.779
2) 5 min. adsorption + ON desorption*	0.245	0.046	0.246	0.173	0.726
3) Overnight adsorption	0.736	0.544	0.680	0.613	0.723

* Overnight desorption in 1% Tween® 20

Although the foregoing invention has been described in some detail by way of illustration and example, for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for adsorbing proteins to a surface, said method comprising:

plasma etching the surface with a gas and under conditions selected to enhance the hydrophobicity thereof; and

adsorbing protein to said plasma-etched surface.

2. A method as in claim 1, wherein the protein is selected from the group consisting of immunoglobulins, avidin, streptavidin, albumins, thyroglobins, and ferritin.

3. A method as in claim 2, wherein the protein is derivatized with a binding hapten selected from the group consisting of biotin, fluorescein, dinitrophenol, digoxin, luminol, theophylline, and morphine.

4. A method as in claim 1, wherein the solid phase surface is composed of an organic polymer.

5. A method as in claim 4, wherein the organic polymer is selected from the group consisting of acrylates, and polystyrenes.

6. A method as in claim 1, wherein the plasma etching is performed with a reactant selected from the group consisting of halocarbon-containing gases, hydrocarbon-containing gases, and halogen-containing gases.

7. A method as in claim 6, wherein the reactant gas comprises a halocarbon selected from the group consisting of CF_4, CCl_4, CF_3Cl, and CF_3Br.

8. A method as in claim 7, wherein the etching is performed for a time in the range from 1 minute to 20 minutes, at a pressure from 0.1 to 1 Torr, and a power of from 100 to 1000 W.

9. A method as in claim 8, wherein the etching is performed in an isotropic etch chamber.

10. A method as in claim 1, wherein the adsorbing step is performed at room temperature for a time between 1 minute and 30 minutes.

11. An improved method for immobilizing protein on a surface, wherein the improvement comprises plasma etching the surface prior to protein immobilization.

12. A method as in claim 11, wherein the protein is selected from the group consisting of immunoglobulins, avidin, streptavidin, albumins, thyroglobins, and ferritin.

13. A method as in claim 12, wherein the protein is derivatized with a binding hapten selected form the group consisting of biotin, fluorescein, dinitrophenol, digoxin, luminol, theophylline, and morphine.

14. A method as in claim 11, wherein the solid phase surface is composed of an organic polymer.

15. A method as in claim 14, wherein the organic polymer is selected from the group consisting of acrylates, and polystyrenes.

16. A method as in claim 11, wherein the plasma etching step is performed with a reactant selected from the group consisting of halocarbon-containing gases, hydrocarbon-containing gases, and halogen-containing gases.

17. A method as in claim 16, wherein the reactant gas is a halocarbon selected from the group consisting of CF_4, CCl_4, CF_3Cl, and CF_3Br.

18. A method as in claim 17, wherein the etching is performed for a time in the range from 1 minute to 20 minutes, at a pressure from 0.1 to 1 Torr, and a power of from 100 to 1000 W.

19. A method as in claim 18, wherein the etching is performed in an isotropic etch chamber.

20. A method as in claim 11, wherein the adsorbing step is performed at room temperature for a time between 1 minute and 30 minutes.

21. An improved test article of the type comprising a protein adsorbed onto a solid phase surface, wherein the improvement comprises a surface which has been plasma etched with a gas and under conditions selected to enhance protein adsorption.

22. An article as in claim 21, wherein the protein is selected from the group consisting of immunoglobulins, avidin, streptavidin, albumins, thyroglobins, and ferritin.

23. An article as in claim 22, wherein the protein is derivatized with a binding hapten selected form the group consisting of biotin, fluorescein, dinitrophenol, digoxin, luminol, theophylline, and morphine.

24. An article as in claim 21, wherein the solid phase surface is composed of an organic polymer.

25. An article as in claim 24, wherein the organic polymer is selected from the group consisting of acrylates, and polystyrenes.

26. An article as in claim 21, wherein the plasma etching step is performed with a reactant selected from the group consisting of halocarbon-containing gases, hydrocarbon-containing gases, and halogen-containing gases.

27. An article as in claim 26, wherein the reactant gas is a halocarbon selected from the group consisting of CF_4, CCl_4, CF_3Cl, and CF_3Br.

28. An article as in claim 27, wherein the etching is performed for a time in the range from 1 minute to 20 minutes, at a pressure from 0.1 to 1 Torr, and a power of from 100 to 1000 W.

29. An article as in claim 28, wherein the etching is performed in an isotropic etch chamber.

30. An article as in claim 21, wherein the improvement further comprises a surface which has been adsorbed with protein at room temperature for a time between 1 minute and 30 minutes.

31. An article as in claim 21, where the protein is immobilized solely by adsorption without covalent attachment.

32. An article as in claim 31, wherein the protein adsorption to the solid phase is sufficiently strong to resist desorption when exposed to 1% Tween® 20 overnight.

33. An improved test article of the type comprising a naturally hydrophilic solid phase surface having fluid flow paths formed thereover, wherein the improvement comprises a fluid flow path which has been plasma etched in order to enhance hydrophobicity to inhibit aqueous flow therethrough.

34. A method for fabricating a test article, said method comprising

providing a naturally hydrophilic solid phase surface having at least one flow path or test chamber therein; and

plasma etching the flow path or test chamber to enhance hydrophobicity.

35. A method as in claim 34, wherein the plasma etching comprises etching the solid phase surface with a halocarbon-containing gas.

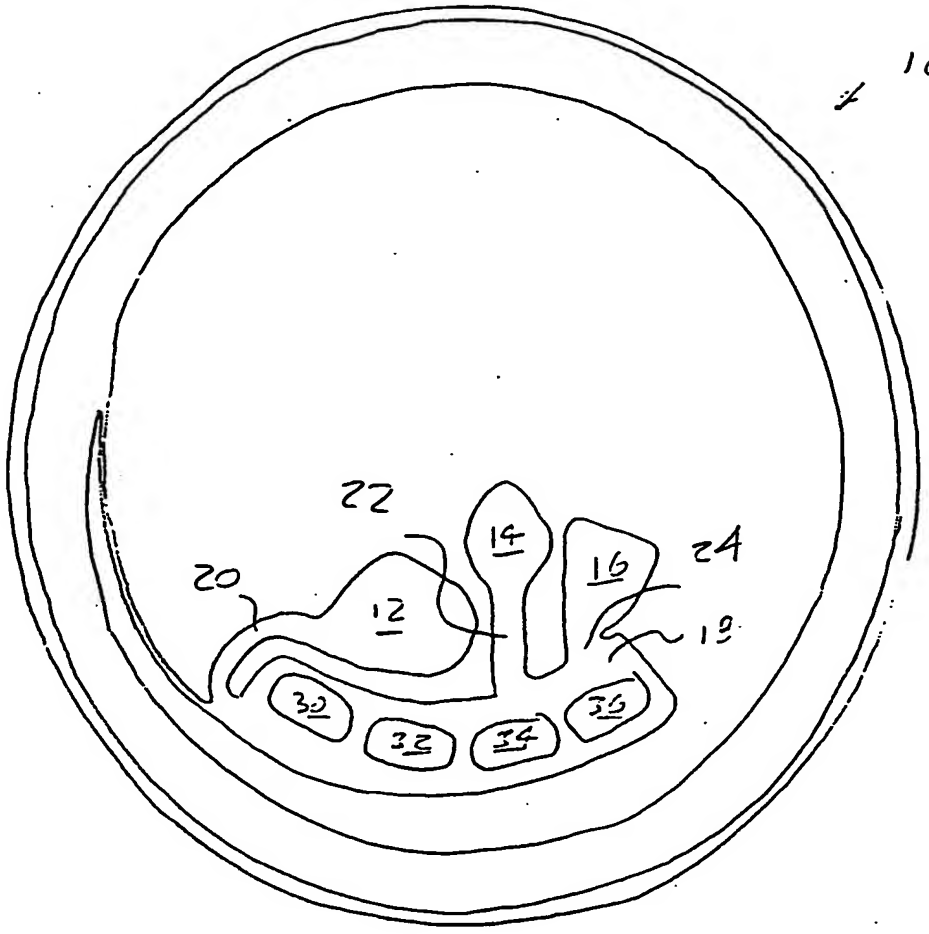

FIG - 1

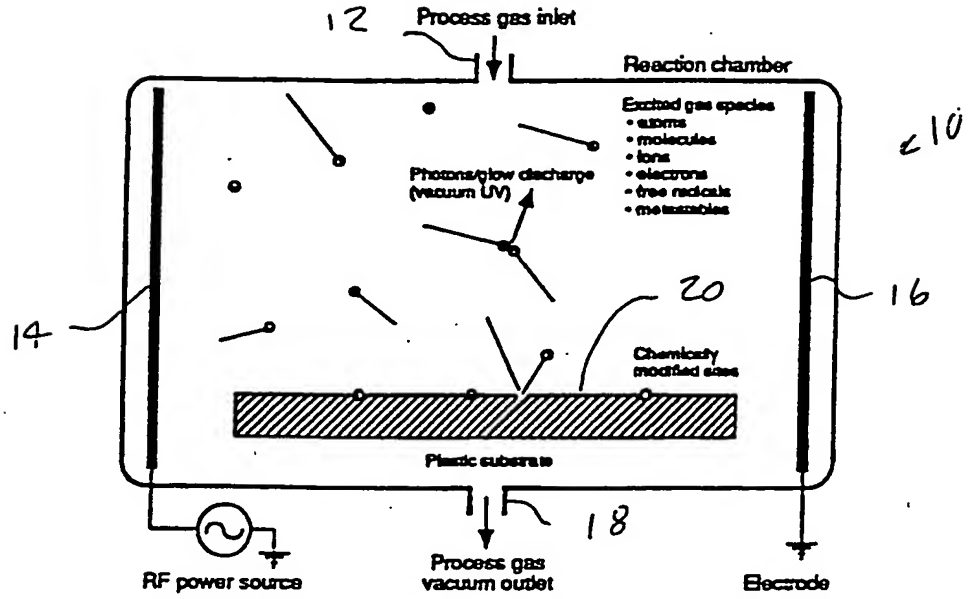
12
Process gas inlet
Reaction chamber
Excited gas species
• atoms
• molecules
• ions
• electrons
• free radicals
• metastables
10
Photons/glow discharge
(vacuum UV)
14
20
16
Chemically
modified sites
Plastic substrate
18
RF power source
Process gas
vacuum outlet
Electrode

FIG- 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/13895

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :B44C 1/22; G01N 33/554, 33/555; C07K 1/00
US CL :156/643, 646; 436/518, 520; 530/350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 156/643, 646; 436/518, 520; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, MEDLINE, CAPLUS, BIOSIS, DERWENT, EMBASE, MEDLINE, BIOTECHIDS, LIFE SCI, CONFSCI, DISSABS, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	US 5,466,575 A (COZZETTE et al.) 14 November 1995, entire document, especially column 15, lines 65-6; column 63, lines 1-5 and 35-41; column 70, lines 35-50; columns 77-78.	1-32
A	LEE. J.H.A wettability gradient as a tool to study protein adsorption and cell adhesion on polymer surfaces. Journal Biomater. Sci. Polymer Edn. 1993. Vol. 4. No. 5. pages 467-481, entire document.	1-32
A	NOJIRI. C. Blood compatibility of PEO grafted polyurethane and HEMA/styrene block copolymer surfaces. Journal of Biomedical Materials Research. 1990. Vol. 24. No. 24. pages 1151-1171, entire document.	1-32
A	US 5,316,784 A (MAURER et al.) 31 May 1994, entire document.	1-32

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 22 OCTOBER 1996	Date of mailing of the international search report 13 NOV 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JENNIFER SHAVER Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/13895

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-32

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.